

WHAT IS CLAIMED IS:

1. A stabilized gas filled microbubble preparation, comprising:

5 a mixture of a first gas or gases and a second gas or gases within generally spherical membranes to form microbubbles, wherein said first gas and said second gas are respectively present in a molar ratio of about 1:100 to about 1000:1, and wherein said first gas has a vapor pressure of at least about $(760 - x)$ mm Hg at 37°C , where x is the vapor pressure of the
10 second gas at 37°C , and wherein said vapor pressure of each of said first and second gases is greater than about 75 mm Hg at 37°C , with the proviso that said first gas and said second gas are not water vapor.

15 2. The preparation of Claim 1, wherein said second gas comprises a fluorocarbon and said first gas is a nonfluorocarbon.

20 3. The preparation of Claim 2, wherein said first gas comprises nitrogen, oxygen, carbon dioxide, or a mixture thereof.

25 4. The preparation of Claim 2, wherein:
said microbubbles are in a liquid medium and have a first average diameter;

the ratio of said first gas to said second gas in said microbubbles is at least 1:1; and

30 said microbubbles are adapted to shrink in said medium as a result of loss of said first gas through said membrane to a second average diameter of less than about 75% of said first diameter and then remain stabilized at or about said second diameter for at least about 1 minute as a result of a gas osmotic pressure differential across said membrane.

5. The preparation of Claim 4, wherein said medium is aqueous.

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6. The preparation of Claim 4, wherein said medium is in a container and said microbubbles have been formed in said container.

5 7. The preparation of Claim 4, wherein said medium is blood *in vivo*.

10 8. The preparation of Claim 4, wherein said liquid medium contains gas or gases dissolved therein with a gas tension of at least about 700 mm Hg, wherein said first diameter is at least about 5 μm , and wherein the tension of the gas or gases dissolved in said medium is less than the pressure of the same gas or gases inside said microbubbles.

15 9. The preparation of Claim 4, wherein said first diameter is at least about 10 μm and said second diameter is between about 1 μm and 6 μm .

10. The preparation of Claim 1, wherein said second gas has an average molecular weight at least about 4 times that of said first gas.

20 11. The preparation of Claim 1, wherein said second gas has a vapor pressure less than about 750 mm Hg at 37°C.

12. The preparation of Claim 11, wherein said molar ratio of said first gas to said second gas is from about 1:10 to about 500:1.

25 13. The preparation of Claim 11, wherein said second gas comprises a fluorocarbon and said first gas is a nonfluorocarbon.

14. The preparation of Claim 13, wherein said second gas comprises at least two fluorocarbons.

15. The preparation of Claim 13, wherein said second gas comprises a perfluorocarbon.

30 16. The preparation of Claim 11, wherein both said first gas and said second gas comprise fluorocarbons.

17. The preparation of Claim 1, wherein said microbubbles contain as said first gas, or as said second gas, or respectively as said first and second gases,

gaseous perfluorobutane and perfluorohexane in a ratio from about 1:10 to about 10:1.

18. The preparation of Claim 1, wherein said microbubbles contain as said first gas, or as said second gas, or respectively as said first and second gases, gaseous perfluorobutane and perfluoropentane in a ratio from about 1:10 to about 10:1.

19. The preparation of Claim 1, wherein said second gas has a water solubility of not more than about 0.5 mM at 25°C and one atmosphere, and wherein said first gas has a water solubility at least about 10 times greater than that of said second gas.

20. The preparation of Claim 1, wherein the permeability of the membrane to said first gas is at least about 5 times greater than the permeability of said membrane to said second gas.

21. The preparation of Claim 1, further comprising:
a container enclosing said microbubbles; and
a liquid in said container in admixture with said microbubbles, wherein said container further comprises means for transmission of sufficient ultrasonic energy to said liquid to permit formation of said microbubbles by sonication.

22. The preparation of Claim 21, wherein said means for transmission comprises a flexible polymer material having a thickness less than about 0.5 mm.

23. The preparation of Claim 1, wherein said membrane is a surfactant.

24. The preparation of Claim 23, wherein said surfactant comprises a non-Newtonian viscoelastic surfactant.

25. The preparation of Claim 23, wherein said surfactant is a carbohydrate.

26. The preparation of Claim 25, wherein said carbohydrate is a polysaccharide.

27. The preparation of Claim 23, wherein said surfactant is a fatty acid ester of a sugar.

28. The preparation of Claim 23, wherein said surfactant is sucrose stearate.

5 29. The preparation of Claim 23, wherein said surfactant is proteinaceous.

30. The preparation of Claim 1, wherein said membrane is solid or semi-solid.

10 31. The preparation of Claim 1, wherein said membrane is a proteinaceous material.

32. The preparation of Claim 30, wherein said proteinaceous material is albumin.

33. A kit for use in preparing microbubbles, comprising:

15 a sealed container;
a liquid in said container;
a surfactant in said container; and
a fluorocarbon gas in said container, wherein
said liquid, said surfactant, and said fluorocarbon
gas or vapor are together adapted to form microbubbles
20 upon the application of energy thereto.

34. The kit of Claim 33, further comprising
means in said container for permitting
transmission of sufficient external ultrasonic energy
25 to said liquid to form microbubbles in said container.

35. The kit of Claim 34, wherein said means for
transmission comprises a flexible polymer membrane having a
thickness less than about 0.5 mm.

36. The kit of Claim 33, further comprising:
a nonfluorocarbon gas in said container, wherein
the molar ratio of said nonfluorocarbon gas to said
fluorocarbon gas is from about 1:10 to about 1000:1,
with the proviso that said nonfluorocarbon gas is not
water vapor.

35 37. A kit for use in preparing microbubbles,
comprising:

a container;

dried liquid-soluble void-containing structures in said container, said void-containing structures defining a plurality of voids having an average diameter less than about 100 μm ;

5 a gas in said voids; and

10 a surfactant, wherein said void-containing structures, said gas, and said surfactant are together adapted to form microbubbles upon addition to said container of a liquid in which said void-containing structures are soluble.

38. The kit of Claim 37, wherein said void-containing structures comprise at least in part said surfactant.

15 39. The kit of Claim 37, wherein said surfactant is a non-Newtonian viscoelastic surfactant.

40. The kit of Claim 37, wherein said surfactant is a fatty acid ester of a sugar.

41. The kit of Claim 37, wherein said surfactant is sucrose stearate.

20 42. The kit of Claim 37, wherein said void-containing structures are proteinaceous.

43. The kit of Claim 37, wherein said void-containing structures are formed of a carbohydrate.

25 44. A method for forming microbubbles, comprising the steps of:

providing a first gas, a second gas, a membrane forming material, and a liquid, wherein said first gas and said second gas are present in a molar ratio of about 1:100 to about 1,000:1, and wherein said first gas has a vapor pressure of at least about $(760 - x)$ mm Hg at 37°C , where x is the vapor pressure of the second gas at 37°C , and wherein said vapor pressure of each of said first and second gases is greater than about 75 mm Hg at 37°C , with the proviso that said first gas and said second gas are not water vapor; and

surrounding said first and second gases with said membrane forming material to form microbubbles in said liquid.

5 45. The method of Claim 44, wherein said membrane forming material is a surfactant.

46. The method of Claim 44, further comprising the steps of:

10 initially forming microbubbles having a first average diameter wherein the initial ratio of said first gas to said second gas in said microbubbles is at least about 1:1;

contacting said microbubbles having a first average diameter with a liquid medium;

15 shrinking said microbubbles in said medium as a result of loss of said first gas through said membrane; and then

20 stabilizing said microbubbles at a second average diameter of less than about 75% of said first diameter for a period of at least one minute.

25 47. The method of Claim 46, wherein said microbubbles are stabilized at said second diameter by:

providing a gas osmotic pressure differential across said membrane such that the tension of a gas or gases dissolved in said medium is equal to or greater than the partial pressure of the same gas or gases inside said microbubbles.

30 48. The method of Claim 47, wherein said first diameter is at least about 5 μm .

49. A method for forming microbubbles, comprising the steps of:

35 providing dried liquid-soluble void-containing structures, said void-containing structures defining a plurality of voids having a diameter less than about 100 μm ;

providing a gas in said voids;
providing a surfactant;

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providing said void-containing structures, said gas, and said surfactant in admixture with a liquid in which said void-containing structures are soluble; and dissolving said void-containing structures in

5 said liquid whereby the gas in said enclosures forms microbubbles that are surrounded by said surfactant.

50. The method of Claim 49, wherein said membrane structures are microspheres.

10 51. The method of Claim 49, wherein said microbubbles contain a first gas and a second gas respectively present in a molar ratio of from about 1:100 to about 1000:1.

52. A method for imaging an object or body, comprising the steps of:

15 introducing into said object or body a microbubble preparation according to Claim 1; and then ultrasonically imaging at least a portion of said object or body.

20 53. The method of Claim 52, wherein said body is a vertebrate and said preparation is introduced into the vasculature of said vertebrate.

54. The method of Claim 52, wherein said preparation is a preparation as defined in Claim 2.

55. The method of Claim 52, wherein said preparation is a preparation as defined in Claim 4.

25 56. The method of Claim 52, wherein said preparation is a preparation as defined in Claim 7.

57. The method of Claim 52, wherein said preparation is a preparation as defined in Claim 10.

30 58. The method of Claim 52, further comprising the step of preparing said microbubble preparation prior to said introduction according to the method of Claim 44.

59. The method of Claim 52, further comprising the step of preparing said microbubble preparation prior to said introduction according to the method of Claim 46.

60. The method of Claim 52, further comprising the step of preparing said microbubble preparation prior to said introduction according to the method of Claim 47.

5 61. The method of Claim 52, further comprising the step of preparing said microbubble preparation prior to said introduction according to the method of Claim 49.

A handwritten diagram illustrating a process flow. It consists of three main stages arranged vertically. The first stage on the left is labeled "add B". An arrow points from "add B" to the second stage in the middle, which is labeled "add C₁". Another arrow points from "add C₁" to the third stage on the right, which is labeled "Add Y+".